Final Report

Original 2 of 2

Determination of the mutagenic potential of

[redacted]

with the in-vitro test

"Chromosome Aberration in Human Lymphocytes"

following OECD 473 and EU B.10

Study No.: [redacted]

Sponsor:

[redacted]

Monitor:

[redacted]

Test Facility:

[redacted]

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1 GLP-COMPLIANCE STATEMENT

It is hereby declared that all tests were made in accordance with the „Revised OECD Principles of Good Laboratory Practice" (Paris, 1997) as stated in the following guidelines:

- OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997; Environment Directorate, Organisation for Economic Cooperation and Development, Paris 1998
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances (codified version)
- Chemikaliengesetz (Chemicals Act) of the Federal Republic of Germany (ChemG) §19 and annexes 1 and 2 in the version of 02 July 2008 Federal Law Gazette, Germany (BGBL) N. 28/2008, pp. 1146-1184, amended in Federal Law Gazette, Germany (BGBL) from 02 November 2011, No. 56/2011, pp. 2162-2169

There were no circumstances that may have affected the quality or integrity of the study.

[redacted]

Study Director

[redacted]

Date

Information on Study Organisation:

Deputy Study Director

Principal Investigator Experimental Phase 2

Study Plan dated

Experimental Starting Date

Experimental Completion Date

Draft Report dated

[redacted]

2 QUALITY ASSURANCE UNIT STATEMENT

This study has been inspected by the quality assurance unit according to the principles of Good Laboratory Practice. Study Plan and Final Report were checked at the dates given below, the Study Director and the management were informed with the corresponding report.

Also, the performance of the study was inspected, and findings were reported to Study Director and management. The inspection of short-term studies (duration less than four weeks) is carried out as audit of process concerning major technical phases of at least one similar test. Frequency is once or more a quarter.

Audit reports of test site QA were written and forwarded in a timely fashion. Content and completeness were checked by lead QA.

The study was conducted and the reports were written in accordance with the Study Plan and the Standard Operating Procedures of the test facility.

Deviations from the Study Plan were acknowledged and assessed by the Study Director and included in the Final Report.

The reported results reflect the raw data of the study.

The quality assurance statement for experimental phase 2 is comprised in the phase study report which is attached to this document.

Verified Procedure	Inspected on	Findings reported on	Audit report no.
Study plan	[redacted]		
Performance of study			
Draft report			
Final report			

[redacted]

Date [redacted]

Quality Assurance Manager

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3 SUMMARY

Title of Study: Determination of the mutagenic potential of [redacted] with the in-vitro test "Chromosome Aberration in Human Lymphocytes" following OECD 473 and EU B.10

Findings and Results:

This study was performed to assess the mutagenic potential of [redacted] to induce structural chromosomal aberrations in human lymphocytes cultured *in vitro* in absence and presence of an exogenous metabolic activation system (liver S9 mix from male rats, treated with Arochlor 1254). The test item was suspended in culture medium without fetal calf serum.

Human lymphocytes, in whole blood culture, were stimulated to divide by addition of phytohaemagglutinin and exposed to the test item both in the presence and absence of S9 mix derived from rat livers. Solvent and positive control cultures were also prepared. Three hours after the end of cultivation, cell division was arrested using Colcemid, the cells were harvested and slides were prepared. Then, the metaphase cells were examined for chromosomal damage.

The following schedule was observed:

Procedure	Exp. I	Exp. II*	Exp. I	Exp. II*
Metabolic activation	Without S9 mix		With S9 mix	
Cell proliferation	72 ± 2 hrs	72 ± 2 hrs	72 ± 2 hrs	72 ± 2 hrs
Exposure period	4 hrs	22 hrs	4 hrs	4 hrs
Recovery	18 hrs	—	18 hrs	18 hrs
Preparation interval	22 hrs	22 hrs	22 hrs	22 hrs
Conc. selected for metaphase analysis	5000, 2500, 1250 µg/mL	4946, 2473, 1236 µg/mL	5000, 2500, 1250 µg/mL	4946, 2473, 1236 µg/mL

* performed for verification purposes.

Two independent experiments were performed. In each experimental group, all cell cultures were set up in duplicates. In order to asses the toxicity of the test solution to cultured human lymphocytes; the mitotic index was calculated for all cultures treated with test item, positive controls and solvent control. On the basis of the data from the mitotic index, the concentrations were selected for metaphase analysis:

In both independent experiments, no toxicity was detected in the treatments without metabolic activation.

In the first experiment, toxicity was detected in the two highest treatments with metabolic activation (5000 and 2500 µg/mL).

In the second experiment, toxicity was detected in the three highest treatments with metabolic activation (4946 µg/mL, 2473 µg/mL and 1236 µg/mL).

In both experiments, no statistically significant and no biologically relevant increase of structural chromosomal aberrations was observed at the evaluated treatments without metabolic activation. Furthermore, no increase in the frequencies of polyploid metaphases was found after treatment with the test item as compared to the frequencies of the controls.

In both experiments, statistically significant and biologically relevant increases of structural chromosomal aberrations were observed at the two highest evaluated treatments with metabolic activation. No increase in the frequencies of polyploid metaphases was found after treatment with the test item as compared to the frequencies of the controls.

All positive control compounds caused large, statistically significant increases in the proportion of aberrant cells, demonstrating the sensitivity of the test system.

In conclusion, under the experimental conditions reported, [redacted] induces structural chromosomal aberrations in human lymphocytes *in vitro*.

4 PURPOSE AND PRINCIPLE OF THE STUDY

The *in vitro* chromosome aberration test is used to screen for possible mammalian mutagens and carcinogens. Many compounds that are positive in this test are mammalian carcinogens; however, there is not a perfect correlation between this test and carcinogenicity. The *in vitro* chromosome aberration test performed in this study is an essential part for genotoxicity studies of substances.

Sponsor's intent: notification according to REACH.

5 PHASES OF THE STUDY

Study Director of the whole study was [redacted] Head of Department Microbiology of [redacted]

The person responsible for the quality assurance of the study was [redacted], Head of Quality Assurance Unit of [redacted]

5.1 Experimental Phase 1

Phase 1 of the study was performed at the test facility [redacted]

Phase 1 included the cell cultivation, cell treatment, the chromosome preparation, the staining of the chromosomes and the evaluation of the mitosis index.

5.2 Experimental Phase 2:

Phase 2 of the study was performed at the test site [redacted]

Phase 2 included structural analysis of metaphases and test evaluation. Principal Investigator for phase 2 was [redacted]

5.3 Communication

All communication between study director and principal investigator or the quality assurance of the test site concerning performance of the study or findings was documented. Print-outs of e-mails were included in the raw data.

6 LITERATURE

The study was conducted in accordance with the following guidelines:

- OECD Guidelines for the Testing of Chemicals, Part 473, adopted 21. Jul. 1997 "In vitro Mammalian Chromosome Abberation Test"
- Council Regulation (EC) No. 440/2008, EU Method B.10: Mutagenicity- In vitro Mammalian Chromosome Aberration Test", dated 30. May 2008

Corresponding SOPs of [redacted]

- [redacted]
- [redacted]

The relevant SOPs of the test site [redacted] are listed in the phase study report.

For evaluation of results, the following literature was used:

- Richardson, C., et al (1989): "Analysis of data from in vitro cytogenetic assays" Kirkland, D.J. (ed.). "Statistical evaluation of mutagenicity test data", Cambridge UniversityPress, Cambridge, 141-154

7 MATERIALS AND METHODS

7.1 Test Item

Designation in Test Facility: [redacted]
Date of Receipt:
Condition at Receipt: room temperature, in proper conditions

7.1.1 Specification

The following information concerning identity and composition of the test item was provided by the sponsor.

[redacted]

Name
Batch no.
Appearance
Composition
CAS No.
EINECS-No.
Molecular formula
Molecular weight
Purity
Homogeneity
Stability
Solubility
Production date
Expiry date
Storage
Hazard information
R-phrases
S-phrases

7.1.2 Storage

The test item was stored in the test facility at room temperature, protected from light.

7.1.3 Preparation

Preparation was the same for both experiments:

The test item was [redacted] in deionised water, ethanol or DMSO. Therefore, the test item was autoclaved and suspended in medium without FCS. A suspension was prepared (containing 2.47 g/50 mL nominal) and shaken for 24 hours. The suspension was centrifuged on the day of the test and the supernatant was used to prepare the geometric series of the concentrations to be tested.

The following treatments (real concentrations) of the test item were prepared in the first experiment:

5000, 2500, 1250, 625, 313, 156, 78 and 39 (all concentrations given in µg/mL).

The following treatments (real concentrations) of the test item were prepared in the second experiment:

4946, 2473, 1236, 618, 309, 155, 77 and 39 (all concentrations given in µg/mL).

7.2 Test System

7.2.1 Specification

Human whole blood treated with anti-coagulant (heparin).

7.2.2 Blood Collection

Blood samples were obtained from healthy donors who neither smoke nor receive medication. The same donor was chosen for both individual experiments:

Table 7.2-a Blood donors

Experimental part	Exposure date	Donor	Examined for chromosomal damage
Experiment I	[redacted]	① male, 35 years	yes ≙ Experiment I with and without S9
Experiment II		① male, 35 years	yes ≙ Experiment II with and without S9

7.3 Solvent Controls

Medium without FCS was used as solvent control for the test item and for the positive control EMS. 0.9 % NaCl was chosen as solvent control for the positive control CPA.

7.4 Positive Controls

The following mutagenic substances were used as positive controls:

7.4.1 Without metabolic activation

Ethyl methanesulphonate (EMS)

CAS no.	62-50-0
Solvent	PBS (phosphate buffered saline)
Supplier:	SIGMA
Purity:	pure
Lot no.:	126K0758
Expiry Date:	23. Jul. 2012
Final concentrations:	400 and 600 µg/mL (Exp. I and Exp. II)

7.4.2 With metabolic activation

Cyclophosphamide (CPA) monohydrate

CAS no.	6055-19-2
Solvent	0.9 % NaCl
Supplier:	SIGMA
Purity:	pure
Lot no.:	097K1569
Expiry Date:	05. Jul. 2012
Final concentration:	35 µg/mL (Exp. I and Exp. II)

7.5 Chemicals and Media

The purity of the chemicals which were used was either "analytical grade" or "for microbiological purposes". All solutions and media were sterilized, either by autoclaving (121 °C, 20 minutes) or by membrane filtration.

All concentrations and volumes are given as nominal concentrations; real weights may differ < 10 %.

7.5.1 Media

Culture base medium RPMI 1640, Supplier Biochrom AG, 12247 Berlin, Germany, serving as base for:

7.5.1.1 Complete Culture medium RPMI 1640

Fetal calf serum	15%
Penicillin/Streptomycin (per mL: 10000 Units Pen./ 10 mg Strep) in H_2O	1%
Phytohaemagglutinin solution in H_2O	4.8 µg/mL

7.5.1.2 Serum free medium RPMI (without fetal calf serum)

Penicillin/Streptomycin (per mL: 10000 Units Pen./ 10 mg Strep) in H_2O	1%
Phytohaemagglutinin solution in H_2O	4.8 µg/mL

7.5.2 Saline G	
NaCl	8000 mg
KCl	400 mg
Glucose x H_2O	1100 mg
Na_2HPO_4 x 7 H_2O	290 mg
KH_2PO_4	150 mg
H_2O demin.	ad 1000 mL
pH is adjusted to 7.2.	
7.5.3 Soerensen buffer	
KH_2PO_4 = Solution A	9.08 g
H_2O demin	ad 1000 mL
Na_2HPO_4 x $2H_2O$ = Solution B	11.88 g
H_2O demin.	ad 1000 mL
Buffer:	
Solution A	254 mL
Solution B	246 mL
pH is adjusted to 6.8, if necessary.	
7.5.4 Giemsa solution 10%	
Giemsa solution; Supplier Merck KGaA	10 mL
Soerensen buffer	ad 100 mL
7.5.5 0.55% KCl solution	
KCL	0.55 g
H_2O demin.	ad 100 mL
7.5.6 Fixans (1:3)	
CH_3COOH	100 mL
CH_3OH	300 mL
7.5.7 Phosphate buffer for S9-Mix	
NaH_2PO_4 * H_2O	3.3111 g
Na_2HPO_4 * $2H_2O$.	31.0 g
H_2O demin.	1000 mL
pH is adjusted to 7.4	
7.5.8 NADP-solution for S9-Mix	
NADP (M_R 765.39 g/mol)	765.39 mg
H_2O demin.	ad 10 mL
7.5.9 Glucose-6-phosphate-solution for S9-Mix	
Glucose-6-phosphat * $2H_2O$ (M_R 340.13 g/mol)	3401.3 mg
H_2O demin.	ad 10 mL
7.5.10 Salt solution for S9-Mix	
KCl	0.7380 g
$MgCl_2$ * $6H_2O$	0.4884 g
H_2O demin.	ad 6 mL

7.5.11 S9-Mix

Phosphate buffer for S9-Mix	22.5 mL
NADP-solution	1 mL
Glu-6-phosphate solution	0.125 mL
Salt solution for S9-Mix	0.5 mL
S9	1 mL

7.5.12 S9

S9 (liver enzyme mixture used for the test with metabolic activation) was not produced by [redacted] but was obtained from a specialized company (Trinova Biochem, Gießen) and stored at – 80°C.

Batch nos:	2827, 2715
Specification:	produced from the livers of male Sprague-Dawley rats which were treated with 500 mg Aroclor 1254/kg body weight intraperitoneally.

7.6 Test Vessels

All vessels used were made of glass or sterilizable plastic. They were sterilized before use in a heating chamber or autoclave. The test was performed in culture flasks. For the transfer of the culture medium or the blood, sterile pipettes and a pipetting automate were used.

Glass measuring flasks and cylinders with conformity sign and standard laboratory material (e.g. Falcon tubes) was also used.

7.7 Instruments and Devices

The following instruments and devices were used in the test.

At test facility [redacted]

- SANYO Labo Autoclave MLS 3020
- Clean bench, category 2 (Axo Safe, MARS 12000) [redacted]
- Pipetting device Accu-Jet
- Incubation chamber [redacted] Binder, 37 °C, 5% CO_2
- Glass thermometer [redacted]
- pH-meter, wtw 340i
- Table water bath GFL, adjustable to 37 °C
- Centrifuge Hettich Universal 320
- Precision Scales PB 5001-SO2 Labostyle 5001
- Precision Scales Mettler Toledo XS6001S
- Analytical scales Mettler Toledo XS205DU [redacted]
- Adjustable pipettes with sterile tips [redacted] 28 (200-2000 µL), 22 (20-200 µL), 20 (1-20 µL))
- Orbital shaker GFL 3005 [redacted]
- Microscopes Zeiss Axiolab and Zeiss AxioLab.A1

Instruments and devices which were used at test site ICD are listed in the phase study report which is attached to this document.

Usage and, if applicable, calibration of all instruments following the corresponding SOP in the current edition.

7.8 Cell Cultivation, Treatment and Preparation

The cell cultivation, treatment and the preparation were performed at the test facility [redacted]

7.8.1 Cell Cultivation

The blood cultures were set up in defined time intervals within 24 hours after collection in 25 cm^2 cell culture flasks for cell proliferation. The following volumes were added to the flask per 10 mL:

- 9 mL complete culture medium RPMI 1640
- 1 mL heparinised whole blood

The cultures were then incubated for 72 hours at 37 °C in a humidified atmosphere with 5 % CO_2.

7.8.2 Cell Treatment

Exposure time with the test item was 4 and 22 hours (see table below).
About 72 ± 2 hrs after seeding, 2 blood cultures for each test group were set up in parallel 25 cm^2 cell culture flasks. The following volumes were added to the flasks per 10 mL (final volume 10 mL in each flask):

Table 7.8-a Culture Contents

Agent	Exposure time		
	4 hours		22 hours
	With S9	Without S9	Without S9
Serum-free medium RPMI	7.5 mL	8 mL	--
Complete culture medium RPMI	--	--	8 mL
S9-Mix	0.5 mL	--	--
blood	1 mL	1 mL	1 mL

To the cell culture, 1 mL solvent control resp. positive control resp. test item suspension (supernatant, see chapter 7.1.3, page 12) was added. Then, the cell cultures were incubated at 37 °C in a humidified atmosphere with 5 % CO_2.

After exposure time 4 hrs, the cells were spun down by gentle centrifugation for 5 minutes. The supernatant containing positive control resp. test item was discarded and the cells were re-suspended in Saline G. The washing procedure was repeated once as described.

After washing, the cells were re-suspended in complete culture medium RPMI 1640 and incubated at 37 °C in a humidified atmosphere with 5 % CO_2 for 18 hours until preparation.

The cultures with exposure time 22 hrs were incubated at 37 °C in a humidified atmosphere with 5 % CO_2 until preparation.

7.8.3 Chromosome Preparation

Preparation was always 22 hrs after exposure initiation. Three hours before harvesting, colcemid was added to the cultures (final concentration: 0.1 µg/mL). Each cell culture was harvested and processed separately for the preparation of chromosomes. 22 hrs after beginning of treatment, the cell cultures were transferred in vials and the cells were spun down by gentle centrifugation (1750 rpm, 10 minutes). The supernatant was discarded and the cells were re-suspended in approximately 10 mL hypotonic solution (0.55 % KCl). Then, the cell suspension was allowed to stand at 37 °C for 15 to 20 minutes. After removal of the hypotonic solution by centrifugation (1750 rpm, 10 minutes), the cell pellet was fixated with a mixture of methanol and glacial acetic acid (3 : 1).

After fixation at 2 – 8 °C, minimum 30 minutes, the cell suspension was spun down by gentle centrifugation (1750 rpm, 10 minutes), the supernatant was discarded and the cell pellet was re-suspended in fixans again. The washing procedures were repeated until the cell pellet was white.

7.8.4 Preparation of Slides

The slides were prepared by dropping the respective cell suspension onto a clean microscope slide. This was performed at the test facility [redacted] The cells were then stained with a 10% solution of Giemsa. All slides, including those of positive and negative controls, were independently coded before microscopic analysis.

7.8.5 Determination of Mitose Index

In all replicates, the mitose index (number of metaphases per 1000 cell nuclei) was determined. From these determinations, the test item concentrations which were evaluated for aberrations were defined.

7.8.6 Transfer of Slides to the Test Site

The microscopical slides were transferred to the test side [redacted] via courier service. Number and designation of transferred slides were documented.

8 PERFORMANCE OF THE STUDY AND FINDINGS

8.1 Overview

The schedule for the experiments is given in the following table.

Table 8.1-a Experimental Set-Up

Procedure	Exp. I	Exp. II*	Exp. I and Exp. II*
Metabolic activation	Without S9 mix		With S9 mix
Cell proliferation	72 ± 2 hrs	72 ± 2 hrs	72 ± 2 hrs
Exposure period	4 hrs	22 hrs	4 hrs
Recovery	18 hrs	—	18 hrs
Preparation interval	22 hrs	22 hrs	22 hrs

*performed because the result of Experiment I was negative.

8.2 Pre-Experiment (Experiment I)

8.2.1 Cytotoxicity Test

Cytotoxicity is characterised by the percentages of mitotic suppression in comparison with the controls by counting 1000 cells per culture in duplicate. The maximum concentration of the test item should be 5 µl/mL, 5 mg/mL or 0.01 mol/l, whichever is the lowest.

In this case, the highest concentration (nominal) was 4940 µg/mL (= 0.01 Mol/L; prepared as suspension, because the test item was not sufficiently soluble).

In the pre-experiment (which could be designated as Experiment I with and without S9), eight concentrations (weighed concentration between 5000 µg/mL and 39 µg/mL) of the test item suspension were used and tested with and without S9. The exposure time was 4 hours and the exposure date [redacted] The preparation interval was 22 hours after start of exposure.

8.2.2 Chromosomal Aberration Assay
Mitotic index determination showed medium toxicity (64 % resp. 44 % inhibition of mitosis) in the two highest treatments (5000 µg/mL and 2500 µg/mL) with metabolic activation (S9). As the supernatant of the suspension was applied, no precipitate was observed.

Where cytotoxicity occurs, the evaluated concentrations should cover a range from the maximum to little or no toxicity. Therefore, the highest concentration 5000 µg/mL and the next two lower concentrations 2500 µg/mL and 1250 µg/mL were selected for structural evaluation.

Structural evaluation was performed in experimental phase 2 and is described in the phase study report which is attached to this document. For each treatment, two cultures were evaluated, analysing 100 cells in each culture.

8.3 Experiment II

8.3.1 Cytotoxicity Test
The test was performed in the same fashion as in experiment I (see previous page). The only exception was the test duration and the choice of a different culture medium in order to ensure vitality of the cells after longer exposure.

In the experimental part without S9, exposure time was 22 hours; the experimental part with S9 was performed as in Experiment I (4 hours exposition, 18 hours recovery). Directly after 22 hours, the preparation started. The exposure date of the test was [redacted]

8.3.2 Chromosomal Aberration Assay
Mitotic index determination showed toxicity in the treatments with metabolic activation (S9) at the following concentrations: 4946 µg/mL, 2473 µg/mL.

As the supernatant of the suspension was applied, no precipitate was observed.

Where cytotoxicity occurs, the evaluated concentrations should cover a range from the maximum to little or no toxicity. Therefore, the highest concentration 4946 µg/mL and the next two lower concentrations 2473 µg/mL and 1236 µg/mL were selected for structural evaluation.

Structural evaluation was performed in experimental phase 2 and is described in the phase study report which is attached to this document. For each treatment, two cultures were evaluated, analysing 100 cells in each culture.

9 RESULTS

9.1 Summary of Results Cytotoxicity

Reduced mitotic indices were observed in the treatment with S9 after 4 hours in the first experiment in the two highest concentrations 5000 and 2500 µg/mL. Reduced mitotic indices were observed in the treatment with S9 after 4 hours in the second experiment, too, in the three highest concentrations: 4946 µg/mL, 2473 µg/mL and 1236 µg/mL.

No reduced mitotic indices were observed in the treatments without S9 after 4 hours resp. 22 hours treatment with test solution (all concentrations). In all experimental parts (with and without S9 mix, incubation for 4 or 22 hours), no precipitation was observed up to the highest required concentration.

9.1.1 Experiment I without S9 - Cytotoxicity

Experiment I without S9 was performed using an exposure period of 4 hours, preparation interval 22 hours. The results of the cytotoxicity test of Experiment I without S9 are presented in the following table:

Table 9.1-a Results Cytotoxicity Experiment I without S9

Concentration	Mitotic indices in % of solvent control
Experiment I: exposure period 4 hrs without S9	
Solvent control culture medium without FCS	100.0 %
Positive control EMS 300 µg/mL	51.2 %
Positive control EMS 600 µg/mL	48.8 %
Test item 5000 µg/mL	80.1 %
Test item 2500 µg/mL	80.3 %
Test item 1250 µg/mL	92.5 %
Test item 625 µg/mL	88.4 %
Test item 313 µg/mL	92.0 %
Test item 156 µg/mL	91.7 %
Test item 78 µg/mL	83.1 %
Test item 39 µg/mL nominal	90.9 %

9.1.2 Experiment I with S9 - Cytotoxicity

Experiment I with S9 was performed using an exposure period of 4 hours, preparation interval 22 hours. The results of the cytotoxicity test of Experiment I with S9 are presented in the following table:

Table 9.1-b Results Cytotoxicity Experiment I with S9

Concentration	Mitotic indices in % of solvent control
Experiment I: exposure period 4 hrs with S9	
Solvent control culture medium without FCS	100.0 %
Solvent control for positive control NaCl 0.9%	100.0 %
Positive control CPA 35 µg/mL	48.4 %
Test item 5000 µg/mL	36.2 %
Test item 2500 µg/mL	56.0 %
Test item 1250 µg/mL	89.9 %
Test item 625 µg/mL	97.7 %
Test item 313 µg/mL	86.2 %
Test item 156 µg/mL	96.3 %
Test item 78 µg/mL	91.3 %
Test item 39 µg/mL	100.5 %

9.1.3 Experiment II without S9 - Cytotoxicity

Experiment II without S9 was performed using an exposure period of 22 hours, preparation interval 22 hours. The results of the cytotoxicity test of Experiment II without S9 are presented in the following table:

Table 9.1-c Results Cytotoxicity Experiment II without S9

Concentration	Mitotic indices in % of solvent control
Experiment II: exposure period 22 hrs without S9	
Solvent control culture medium without FCS	100.0 %
Positive control EMS 300 µg/mL	39.8 %
Positive control EMS 600 µg/mL	23.6 %
Test item 4946 µg/mL	87.3 %
Test item 2473 µg/mL	111.6 %
Test item 1236 µg/mL	104.2 %
Test item 618 µg/mL	126.2 %
Test item 309 µg/mL	145.2 %
Test item 155 µg/mL	98.1 %
Test item 77 µg/mL	109.7 %
Test item 39 µg/mL	87.3 %

9.1.4 Experiment II with S9 - Cytotoxicity

Experiment II with S9 was performed using an exposure period of 4 hours, preparation interval 22 hours. The results of the cytotoxicity test of Experiment II with S9 are presented in the following table:

Table 9.1-d Results Cytotoxicity Experiment II with S9

Concentration	Mitotic indices in % of solvent control
Experiment II: exposure period 4 hrs with S9	
Solvent control culture medium without FCS	100.0 %
Solvent control for positive control NaCl 0.9%	100.0 %
Positive control CPA 35 µg/mL	32.6 %
Test item 4946 µg/mL	15.2 %
Test item 2473 µg/mL	32.6 %
Test item 1236 µg/mL	58.3 %
Test item 618 µg/mL	84.4 %
Test item 309 µg/mL	83.0 %
Test item 155 µg/mL	73.2 %
Test item 78 µg/mL	87.7 %
Test item 39 µg/mL	84.1 %

9.2 Summary of Results Genotoxicity

9.2.1 Experiment I – Genotoxicity

Biologically relevant and statistically significant increases of structural chromosomal aberrations was detected in the two highest treatments with metabolic activation. Details are described in the phase study report which is attached to this document.

The results of Experiment I are presented in the following table:

Table 9.2-a Results Genotoxicity Experiment I

Concentration	Aberrant cells in %		
	Inclusive gaps*	Exclusive gaps*	with exchanges
Experiment I: exposure period 4 hrs without S9			
Solvent control culture medium without FCS	4.0	1.0	0
Positive control EMS 600 µg/ml	41.0	**38.5** [s]	18.5
Test item 5000 µg/ml	3.5	2.0	0
Test item 2500 µg/ml	4.5	1.0	0
Test item 1250 µg/ml	4.0	0.5	0
Experiment I: exposure period 4 hrs with S9			
Solvent control medium without FCS 0.5%	2.0	0.5	0
Solvent NaCl 0.9%	1.5	1.0	0
Positive control CPA 35 µg/ml	46.0	**36.0** [s]	6.0
Test item 5000 µg/ml	18.0	**13.0** [s]	1.5
Test item 2500 µg/ml	12.5	**7.0** [s]	1.5
Test item 1250 µg/ml	3	0.5	0

*** Inclusive cells carrying exchanges**
[s] Aberration frequency statistically significant higher than corresponding control values

A second experiment (Experiment II) was performed for verification purposes.

Note: in this table, only the mean values are stated. Detailed data is given in the phase study report which is attached to this document.

9.2.2 Experiment II - Genotoxicity

Biologically relevant and statistically significant increases of structural chromosomal aberrations was detected in the two highest treatments with metabolic activation. Details are described in the phase study report which is attached to this document.

The results of Experiment II are presented in the following table:

Table 9.2-b Results Genotoxicity Experiment II

Concentration	Aberrant cells in %		
	Inclusive gaps*	Exclusive gaps*	with exchanges
Experiment II: exposure period 22 hrs without S9			
Solvent control culture medium without FCS	4.5	2.5	0
Positive control EMS 400 µg/mL	32.0	**25.5** [S]	2.0
Test item 4946 µg/ml	1.5	1.0	0
Test item 2473 µg/ml	1.5	0.5	0
Test item 1236 µg/ml	1.0	0	0
Experiment II: exposure period 4 hrs with S9			
Solvent control culture medium without FCS 0.5%	5.5	1.0	0
Solvent NaCl 0.9%	3.0	0.5	0
Positive control CPA 35 µg/mL	44.0	**38.0** [S]	5.5
Test item 4946 µg/ml	35.5	**32.0** [S]	9.0
Test item 2473 µg/ml	17.0	**13.0** [S]	1.0
Test item 1236 µg/ml	2.5	1.0	0
Test item 618 µg/ml	4.0	1.0	0

*** Inclusive cells carrying exchanges**
[S] Aberration frequency statistically significant higher than corresponding control values

Note: in this table, only the mean values are stated. Detailed data is given in the phase study report which is attached to this document.

10 Discussion and Conclusion

The test item is considered as cytotoxic when applied with metabolic activation down to a concentration of approximately 1250 µg/mL: The test item showed toxicity in both experiments in the experimental part with metabolic activation (S9). In the first experiment, ,toxicity was observed in the two highest concentrations (5000 and 2500 µg/mL). In the second experiment, toxicity was observed in the three highest concentrations (4946 µg/mL, 2473 µg/mL, and 1236 µg/mL)

Therefore, the highest concentration to be evaluated for induction of chromosomal damage was the maximum concentration of 0.01 mol/L.

The test item is considered as genotoxic, because a biologically relevant and statistically significant increase of structural chromosome aberrations was observed in the two highest evaluated concentrations (with metabolic activation). The observed increase showed a concentration-effect relationship

Solvent control and positive controls showed numbers within the range of the historical data and met the acceptability criteria.

No precipitation of the test item was observed, as the supernatant of a suspension was applied.

No observations were made which might cause doubts concerning the validity of the study outcome.

In conclusion, it can be stated that under the experimental conditions reported, the test item [redacted] induces structural chromosomal aberrations in human lymphocytes in vitro in the selected concentrations.

11 DEVIATIONS

11.1 Deviations from the Study Plan

No deviations from the study plan were observed.

11.2 Deviations from the Guideline

No deviations were observed.

12 RECORDING

One original of study plan and final report, respectively, all raw data of the study and all documents mentioned or referred to in study plan or final report will be kept in the GLP Document Archive of the test facility for fifteen years. After that, the sponsor's instructions will be applied (shipment of documentation to sponsor). A retain sample of the test item will be kept in the GLP Substance Archive for fifteen years; then, the retain sample will be discarded.

Number of originals which will be sent to the sponsor: 1

13 ANNEX 1: COPY OF GLP-CERTIFICATE

RheinlandPfalz

LANDESAMT FÜR UMWELT, WASSERWIRTSCHAFT UND GEWERBEAUFSICHT

GUTE LABORPRAXIS – GOOD LABORATORY PRACTICE

GLP-BESCHEINIGUNG
STATEMENT OF GLP COMPLIANCE

gemäß/according to § 19b Abs. 1 Chemikaliengesetz

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EC at:

Prüfeinrichtung / Test facility

[redacted]

Prüfung nach Kategorien / Areas of Expertise

(gemäß / according ChemVwV-GLP Nr. 5.3/OECD guidance)

1, 3, 4, 5, 6 , 8, 9 (toxikologische in Vitro Prüfungen an Säugerzellen und Bakterien)

Datum der Inspektion / Date of Inspection

(Tag.Monat.Jahr / day.month.year)

29. und 30. November 2010

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that the test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Eine erneute behördliche Überprüfung der Einhaltung der GLP-Grundsätze durch die Prüfeinrichtung ist so rechtzeitig zu beantragen, dass die Folgeinspektion spätestens vier Jahre nach dem Beginn der o.g. Inspektion stattfinden kann. Ohne diesen Antrag wird die Prüfeinrichtung nach Ablauf der Frist aus dem deutschen GLP-Überwachungsprogramm genommen und diese GLP-Bescheinigung verliert ihre Gültigkeit.

Verification of the compliance of the test facility with the Principles of the GLP has to be applied for in time to allow for a follow-up inspection to take place within four years after commencing the above mentioned inspection. Elapsing this term, the test facility will be taken out of the German GLP-Monitoring Programme and this GLP Certificate becomes invalid.

Unterschrift, Datum / Signature, Date

24.04.09

Dr.-Ing. Stefan Hill - Präsident -

(Name und Funktion der verantwortlichen Person / name and function of responsible person)

Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht
Kaiser-Friedrich-Straße 7, 55116 Mainz

(Name und Adresse der GLP-Überwachungsbehörde / Name and adress of the GLP Monitoring Authority)

MESSEN BEWERTEN BERATEN

14 ANNEX 2: EXPERIMENT I – DETAILED DATA

14.1 Toxicity

The mitotic index was determined in two parallel cultures (culture 1 and culture 2), 1000 cells per culture of each test group were evaluated. Mitotic index is given in %.
In the table below, mitotic

Table 14.1-a Mitotic Indices Experiment I without Metabolic Activation

Concentration / test group	Mitotic index in %			Mitotic index in % of solvent control
	Culture 1	Culture 2	Mean	
Experiment I: exposure period 4 hrs without S9, exposure date [redacted]				
Solvent control	17.8	18.3	18.0	100 %
Positive control EMS 400 µg/mL	9.7	8.8	9.3	51.2 %
Positive control EMS 600 µg/mL	8.4	9.2	8.8	48.8 %
Test item 5000 µg/mL	14.5	14.4	14.5	80.1 %
Test item 2500 µg/mL	14.9	14.1	14.5	80.3 %
Test item 1250 µg/mL	17.9	15.5	16.7	92.5 %
Test item 625 µg/mL	14.9	17.0	16.0	88.4 %
Test item 313 µg/mL	17.8	15.4	16.6	92.0 %
Test item 156 µg/mL	15.6	17.5	16.6	91.7 %
Test item 78 µg/mL	14.7	15.3	15.0	83.1 %
Test item 39 µg/mL	16.2	16.6	16.4	90.9 %

Table 14.1-b Mitotic Indices Experiment I with Metabolic Activation

Concentration / test group	Mitotic index in %			Mitotic index in % of solvent control
	Culture 1	Culture 2	Mean	
Experiment I: exposure period 4 hrs with S9, exposure date [redacted]				
Solvent control culture medium without FCS	10.6	11.2	10.9	100 %
Solvent control for positive control 0.9 % NaCl	9.1	9.5	9.3	100 %
Positive control CPA 35 µg/mL	5.2	3.8	4.5	32.6%
Test item 5000 µg/mL	4.4	3.5	4.0	36.2 %
Test item 2500 µg/mL	6.2	6.0	6.1	56.0 %
Test item 1250 µg/mL	10.4	9.2	9.8	89.9 %
Test item 625 µg/mL	10.3	11.0	10.7	97.7 %
Test item 312 µg/mL	9.7	9.1	9.4	86.2 %
Test item 156 µg/mL	11.1	9.9	10.5	96.3 %
Test item 78 µg/mL	10.0	9.9	10.0	91.3 %
Test item 39 µg/mL	11.6	10.3	11.0	100.5 %

15 ANNEX 3: EXPERIMENT II – DETAILED DATA

15.1 Toxicity

The mitotic index was determined in two parallel cultures (culture 1 and culture 2), 1000 cells per culture of each test group were evaluated. Mitotic index is given in %.

Table 15.1-a Mitotic Indices Experiment II without Metabolic Activation

Concentration / test group	Mitotic index in %			Mitotic index in % of solvent control
	Culture 1	Culture 2	Mean	
Experiment II: exposure period 22 hrs without S9, exposure date [redacted]				
Solvent control	12.5	13.4	13.0	100 %
Positive control EMS 400 µg/mL	5.5	4.8	5.2	39.8 %
Positive control EMS 600 µg/mL	3.2	2.9	3.1	23.6 %
Test item 4946 µg/mL	12.1	10.5	11.3	87.3 %
Test item 2473 µg/mL	15.1	13.8	14.5	111.6 %
Test item 1236 µg/mL	14.1	12.9	13.5	104.2 %
Test item 618 µg/mL	16.2	16.6	16.4	126.6 %
Test item 309 µg/mL	19.2	18.4	18.8	145.2 %
Test item 155 µg/mL	13.0	12.4	12.7	98.1 %
Test item 78 µg/mL	13.6	14.8	14.2	109.7 %
Test item 39 µg/mL	11.4	11.2	11.3	87.3 %

Table 15.1-b Mitotic Indices Experiment II with Metabolic Activation

Concentration / test group	Mitotic index in %			Mitotic index in % of solvent control
	Culture 1	Culture 2	Mean	
Experiment II: exposure period 4 hrs with S9, exposure date [redacted]				
Solvent control culture medium without FCS	14.4	13.2	13.8	100 %
Solvent control for positive control 0.9 % NaCl	12.5	14.1	13.3	100 %
Positive control CPA 35 µg/mL	4.8	5.2	5.0	32.6%
Test item 4946 µg/mL	2.4	1.8	2.1	15.2 %
Test item 2473 µg/mL	4.8	4.2	4.5	32.6 %
Test item 1236 µg/mL	7.2	8.9	8.1	58.3 %
Test item 618 µg/mL	11.3	12.0	11.7	84.4 %
Test item 309 µg/mL	12.0	10.9	11.5	83.0 %
Test item 155 µg/mL	9.9	10.3	10.1	73.2 %
Test item 78 µg/mL	13.2	11.0	12.1	87.7 %
Test item 39 µg/mL	12.1	11.1	11.6	84.1 %

16 ANNEX 4: PHASE STUDY REPORT

The Phase Study Report consists of 27 pages which are attached to this document.

Phase Report [redacted]

Study No.: [redacted]

Test Item: [redacted]

Phase Report [redacted]

Original 2 of 2

Determination of the mutagenic potential of [redacted]

with the in-vitro test
"Chromosome Aberration in Human Lymphocytes"
following OECD 473 and EU B.10

Experimental Phase 2:

Chromosome analysis and test evaluation

Study No.: [redacted]

[redacted]

1 GLP-Compliance Statement

It is hereby declared that all tests were made in accordance with the „Revised OECD Principles of Good Laboratory Practice" (Paris, 1997) as stated in the following guidelines:

- OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997; Environment Directorate, Organisation for Economic Cooperation and Development, Paris 1998
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances (codified version)
- Chemikaliengesetz (Chemicals Act) of the Federal Republic of Germany (ChemG) §19a and §19b and annexes 1 and 2 version 02 July 2008, Federal Law Gazette, Germany (BGBL) N. 28/2008, pp. 1146-1184, amended in Federal Law Gazette, Germany (BGBL) from 02 November 2011, No. 56/2011, pp. 2162-2169

Responsibility for the accuracy of the information concerning the test item as well as for its authenticity rests with the sponsor.

I herewith accept responsibility for the data presented within this report.

There were no circumstances that may have affected the quality or integrity of the study.

[redacted]

Information on Study Organisation:

[redacted]

Phase Study Plan dated

Experimental phase 2 starting date

Experimental phase 2 completion date

Draft Phase Report dated

2 QUALITY ASSURANCE UNIT STATEMENT

This study has been inspected by the quality assurance unit according to the principles of Good Laboratory Practice. Phase Study Plan and Final Phase Report were checked at the dates given below; the Study Director and the management were informed with the corresponding report.

Also, the performance of the study was inspected, and findings were reported to Study Director and management. The inspection of short-term studies (duration less than four weeks) is carried out as audit of process concerning major technical phases of at least one similar test. Frequency is once or more a quarter.

The study was conducted and the reports were written in accordance with the Phase Study Plan and the Standard Operating Procedures of the test facility.

Deviations from the Phase Study Plan were acknowledged and assessed by the Study Director and included in the Final Phase Report.

The reported results reflect the raw data of the study

Verified Procedure	Inspected on	Findings reported on	Audit report no.
Phase Study plan	[redacted]		
Performance of study			
Draft Phase report			
Final Phase report			

[redacted]

Table of Contents

3 SUMMARY

The study was performed to assess the mutagenic potential of [redacted] to induce structural chromosomal aberrations in human lymphocytes cultured *in vitro* in absence and presence of an exogenous metabolic activation system (liver S9 mix from male rats, treated with Aroclor 1254).

Two independent experiments were performed. In order to assess the toxicity of [redacted] to cultured human lymphocytes, the mitotic index was calculated for all cultures treated with the test substance and the solvent control. On the basis of these data, the following concentrations were selected for metaphase analysis:

Experiment I

Without S9 mix / 4 hour exposure / 18 hour recovery: 5000, 2500 and 1250 µg/mL

With S9 mix / 4 hour exposure / 18 hour recovery: 5000, 2500 and 1250 µg/mL

Experiment II

Without S9 mix / 22 hour exposure / no recovery: 4946, 2473 and 1236 µg/mL

With S9 mix / 4 hour exposure / 18 hour recovery: 4946, 2473, 1236 and 618 µg/mL

In the absence of S9 mix, in both independent experiments, neither a statistically significant nor a biologically relevant increase of structural chromosomal aberrations was observed at the evaluated concentrations.

Only in the presence of S9 mix [redacted] caused statistically significant ($p < 0.001$, excluding gaps) increases of structural chromosomal aberration at the following concentrations (nominal): 2500 resp. 2470 and 5000 resp. 4940 µg/mL, when compared with the solvent control. This effect was found in both independent experiments.

An increase in the frequencies of polyploid metaphases was not found after treatment with the test item as compared to the frequencies of the controls.

All positive controls caused large, statistically significant increases in the proportion of aberrant cells, demonstrating the sensitivity of the test system.

In conclusion, in the human chromosome aberration test, [redacted] can declared as promutagen, it shows evidence of mutagenic potential in presence of the exogenous metabolic activation system (S9 mix) *in vitro*. Under the experimental conditions with S9 mix described, the test item triggered clastogenic activity. It also noted that the toxic and clastogenic effects do not appear in absence of the exogenous metabolic activation system (without S9 mix) at the evaluated concentrations in human lymphocytes *in vitro*.

4 INTRODUCTION AND PURPOSE

The *in vitro* chromosome aberration test is used to screen for possible mammalian mutagens and carcinogens. Many compounds that are positive in this test are mammalian carcinogens; however, there is not a perfect correlation between this test and carcinogenicity.

This study was performed in order to evaluate the mutagenic potential of [redacted] to induce structural chromosomal aberrations in human lymphocytes in absence and presence of a exogenous metabolic activation system (liver S9 mix from male rats, treated with Aroclor 1254). The cytogenetic damage will be determined. The *in vitro* chromosome aberration test is an essential part for genotoxcity studies of substances.

The study was performed the following purpose: Registration in accordance with REACH.

5 LITERATURE

The study was conducted in accordance with the following guidelines:

- OECD Guidelines for the Testing of Chemicals, Part 473, adopted 21. Jul. 1997 "In vitro Mammalian Chromosome Abberation Test"
- Council Regulation (EC) No. 440/2008, EU Method B.10: Mutagenicity- In vitro Mammalian Chromosome Aberration Test", dated 30. May 2008
- ISCN 2009 An International System for Human Cytogenetic Nomenclature (2011), L.G. Shaffer, M.L. Slovak, L.J. Campbell (Eds). S. Karger AG, Basel.

Corresponding SOPs of [redacted]

- [redacted]
- [redacted]

For evaluation of results, the following literature was used:

- Richardson, C., et al (1989): "Analysis of data from in vitro cytogenetic assays" Kirkland, D.J. (ed.). "Statistical evaluation of mutagenicity test data", Cambridge UniversityPress, Cambridge, 141-154

6 MATERIALS AND METHODS

6.1 Chemicals and Devices

The chromosomes slides were analysed with oil microscopy.

The following instruments were used in the test.

- Microscopes Leica DMLB2 and Leica DMLM, Objectives: 10 x and 100 x; oil immersion
- tally counters Nos. 1 and 2

Usage and, if applicable, calibration of all instruments following the corresponding SOP in the current edition.

6.2 Evaluation Procedure

6.2.1 Analysis of Metaphase Cells

All slides were coded. For the analysis of chromosomal aberrations, 100 well spread metaphases per evaluated culture were scored.

Evaluation of the slides was performed using Leica microscopes with 100 x oil immersion objectives. Breaks, fragments, deletions, exchanges and chromosomal disintegrations were recorded as structural chromosome aberrations. Gaps were recorded separately and reported, but they were not included in the total aberration frequency. Chromosome aberrations were scored according to the classification of ISCN 2009. At least 100 well spread metaphases per culture were scored for cytogenetic damage. Only metaphases with 46 ± 1 centromer regions were included in the analysis.

The number of aberrant metaphase cells in each treatment group was compared with the solvent control value using Fisher's exact test.

6.2.2 Data Recording

The generated data were recorded on raw data sheets. The results were presented in tabular from, including experimental groups with the test item, solvent controls and positive controls.

7 PERFORMANCE OF THE STUDY AND FINDINGS

7.1 Evaluation

On the basis of the mitotic index (performed within experimental phase 1), no cytotoxicity was observed in Experiment I and II without S9, therefore, the highest test concentration of [redacted] to human blood cell culture *in vitro* was 5 mg/mL (nominal concentration; the supernatant of suspensions was used). On the basis of the miotic index of the Experiment I and II in the presence of S9 cytotoxicity occurse, so that the highest chosen concentration caused a significant reduction of the mitose index greater than 50% of the solvent control value.

According to OECD 473, evaluated concentrations for cytogenetic damage should be divided by a factor between 2 and √10.

The following concentrations for analysis of cytogenetic damage were chosen:

Experiment I:

Without S9 mix / 4 hour exposure / 18 hour recovery: 5000, 2500 and 1250 µg/mL
With S9 mix / 4 hour exposure / 18 hour recovery: 5000, 2500 and 1250 µg/mL
Experiment II:
Without S9 mix / 22 hour exposure / no recovery: 4946, 2473 and 1236 µg/mL
With S9 mix / 4 hour exposure / 18 hour recovery: 4946, 2473, 1236 and 618 µg/mL

7.2 Metaphase Analysis Experiment I

In Experiment I, significant toxic effects compared with the solvent control were only observed in the experimental part with metabolic activation (S9) after 4 hours treatment with 2500 µg test item/mL and above. Furthermore, this experimental part showed statistically significant increases in the proportion of cells with chromosomal aberrations at 2500 and 5000 µg/mL ($p < 0.001$, excluding gaps), when compared with the solvent control.

In the experimental part without metabolic activation, no cytotoxic effects were observed at all treatments and no relevant increase of structural chromosomal aberrations was detected at the evaluated concentrations. A second experiment (Experiment II) was performed for verification purposes. Details see chapter 13, page 19.

7.3 Metaphase Analysis Experiment II

The Experiment II confirmed the findings from Experiment I: Only in the experimental part with metabolic activation (S9), cytotoxic effect at 2473 µg test item/mL and above were detected. A significant increase of structural chromosome aberrations was observed in treatments 2473 and 4946 µg/mL ($p < 0.001$, excluding gaps), when compared with the solvent control.

In the experimental part without metabolic activation, neither toxic effects nor relevant increase of structural chromosomal aberrations after treatment with the test item at the evaluated concentrations were observed. Details see chapter 14, page 21.

[redacted] shows the characteristic behaviour of a promuatgen.

8 RESULTS

8.1 Summary of Results

Experiment I was performed using an exposure period of 4 hours, preparation interval 22 hours.

The results of Experiment I are presented in the following table:

Table 8.1-a Results Experiment I

Concentration	Aberrant cells in %		
	Inclusive gaps*	Exclusive gaps*	with exchanges
Experiment I: exposure period 4 hrs without S9			
Solvent control: 0.5% (v/v) Culture medium without FCS#	4.0	1.0	0
Positive control: 600 µg/mL EMS	41.0	**38.5 S**	18.5
Test item: 5000 µg/mL [redacted]	3.5	2.0	0
Test item: 2500 µg/mL [redacted]	4.5	1.0	0
Test item 1250 µg/mL [redacted]	4.0	0.5	0
Experiment I: exposure period 4 hrs with S9			
Solvent control: 0.5% (v/v) Culture medium without FCS#	2.0	0.5	0
Solvent control of the positive control: 0.5% NaCl (0.9%)	1.5	1.0	0
Positive control: 35 µg/mL CPA	46.0	**36.0 S**	6.0
Test item: 5000 µg/mL [redacted]	18.0	**13.0 S**	2.0
Test item: 2500 µg/mL [redacted]	12.5	**7.0 S**	1.5
Test item 1250 µg/mL [redacted]	3	0.5	0

*** Inclusive cells carrying exchanges**
 # FCS = fetal calf serum
S Aberration frequency statistically significant higher than corresponding control values

Experiment II was performed using an exposure period of 22 hours without S9 and 4 hours with S9, preparation interval 22 hours.

The results of Experiment II are presented in the following table:

Table 8.1-b Results Experiment II

Concentration	Aberrant cells in %		
	Inclusive gaps*	Exclusive gaps*	with ex-changes
Experiment II: exposure period 22 hrs without S9			
Solvent control: 0.5% (v/v) Culture medium without FCS#	4.5	2.5	0
Positive control: 300 µg/mL EMS	32.0	**25.5** S	2.0
Test item: 4946 µg/ml [redacted]	1.5	1.0	0
Test item: 2473 µg/ml [redacted]	1.5	0.5	0
Test item 1236 µg/mL [redacted]	1.0	0	0
Experiment II: exposure period 4 hrs with S9			
Solvent control: 0.5% (v/v) Culture medium without FCS#	5.5	1.0	0
Solvent control of the positive control: 0.5% NaCl (0.9%)	3.0	0.5	0
Positive control: 35 µg/mL CPA	44.0	**38.0** S	5.5
Test item: 4946 µg/ml [redacted]	35.5	**32.0** S	8.5
Test item: 2473 µg/ml [redacted]	17.0	**13.0** S	1.0
Test item 1236 µg/mL [redacted]	2.5	1.0	0
Test item 618 µg/ml [redacted]	4.0	1.0	0

* Inclusive cells carrying exchanges
# FCS = fetal calf serum
S Aberration frequency statistically significant higher than corresponding control values

8.2 Structural Aberrations

For the analysis of chromosomal aberrations, 100 well spread metaphases per evaluated culture were scored.

In this study, in both independent experiments, no biologically relevant increase of structural chromosomal aberrations was observed in the absence of S9 mix. The range of aberrant cells after treatment with the test item (0.5 – 2% aberrant cells, excluding gaps) was not significantly higher than the range of the solvent control values (0.5 – 2.5% aberrant cells, excluding gaps).

In both independent experiments, a biologically relevant increase of structural chromosomal aberrations was observed in the presence of S9 mix. The range of aberrant cells after treatment with the test item (0.5 – 32% aberrant cells, excluding gaps) was significantly higher than the range of the solvent control values (0.5 – 1% aberrant cells, excluding gaps)

Detailed data is presented in the annexes (Experiment I: chapter 13.1, page 19; Experiment II: chapter 14.1, page 21).

No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.

In both experiments, EMS (600 µg/mL and 300 µg/mL, respectively) and CPA (35 µg/mL) were used as positive controls and showed distinct increases in cells with structural chromosome aberrations. EMS was evaluated in a lower concentration in experiment II, as exposure period was much longer.

8.3 Acceptability

The assay is considered acceptable as it meets the following criteria:

- In the **solvent control** the number of cells with aberrations (aberrant cells excl. gaps) must be between 0 – 5.0 %.
- In the **positive controls** (EMS / CPA) the number of cells with aberrations (aberrant cells excl. gaps) must be higher than ≥ 5.0 %.

In the study, the following data was obtained.

Table 8.3-a Acceptability Criteria

	Aberrant cells [%] excl. gaps		
	Solvent Control (culture medium)	**Positive control (EMS)**	**Positive control (CPA)**
Target Value	0 – 5.0	≥ 5.0	≥ 5.0
Experiment			
I / without S9	1.0	38.5	--
I / with S9	0.5	--	36.0
II / without S9	1.5	25.5	--
II / with S9	1.0	--	38.0

All target values were met.

8.4 Classification

A test item is classified as **non-mutagenic** if:

- the number of induced structural chromosome aberrations in all evaluated dose groups is not above 5.0 % aberrant cells, excluding gaps.
- no significant increase of the number of structural chromosome aberrations is observed.

A test item is classified as **mutagenic** if:

- the number of induced structural chromosome aberrations is above 5.0 % aberrant cells, excluding gaps.
- and either a concentration-related or a significant increase in the number of cells with structural chromosome aberrations is observed.

A test item is classified as **promutagenic** if:

- an increase of structural chromosome aberrations is only observed in the experimental parts with metabolic activation system (S9 mix). In the presence of S9 mix:
  - the number of induced structural chromosome aberrations is above 5.0 % aberrant cells, excluding gaps.
  - and either a concentration-related or a significant increase in the number of cells with structural chromosome aberrations is observed

The recorded data in the study declare the test item [redacted] as a promutagen. Under the experimental conditions described, the test item triggered clastogenic activity in the presence of a metabolic activation system (S9). Without metabolic activation, no increase of structural chromosomal aberrations was observed at the evaluated concentrations.

Statistical significance was confirmed by means of the Fisher's exact test (see chapter 15, page 23).

9 DISCUSSION AND CONCLUSION

In two independent experiments, the test item [redacted] showed mutagenic effects in the presence of a metabolic activation system (S9) in treatments with concentrations of 2500 µg/mL (nominal) and above; statistically significant and biologically relevant increases of structural chromosome aberrations were found.

Solvent control and positive controls showed numbers within the range of the historical data and met the acceptability criteria.

No observations were made which might cause doubts concerning the validity of the study outcome.

In conclusion it can be stated that under the experimental conditions reported [redacted] shows evidence mutagenic potential in presence of the exogenous metabolic activation system (S9 mix) *in vitro*. Under the experimental conditions with S9 mix described, the test item triggered clastogenic activity. It also noted that the toxic and clastogenic effects do not appear in absence of the exogenous metabolic activation system (without S9 mix) at the evaluated concentrations in human lymphocytes *in vitro*. The recorded data in the study declare the test item [redacted] as a pro-mutagen.

10 DEVIATIONS

10.1 Deviations from the Study Plan

No deviations from the study plan were observed.

10.2 Deviations from the Guideline

No deviations from the guideline were observed.

11 RECORDING

One original of study plan and final report, respectively, all raw data of the study and all documents mentioned or referred to in study plan or final report will be kept in the GLP Document Archive of the test facility for fifteen years. After that, the sponsor's instructions will be applied (shipment of documentation to sponsor). A retain sample of the test item will be kept in the GLP Substance Archive for fifteen years; then, the retain sample will be discarded.

Number of originals which will be sent to the sponsor: 1.

12 ANNEX 1: CHROMOSOME ABERRATIONS: CLASSIFICATION AND CRITERIA

A chromatid aberration involves only one chromatid in a chromosome at a given locus.

12.1 Chromatid gaps (g) and chromosome gap (ig)

A chromatid gap is a non-staining region (achromatic lesion) of a single chromatid in which there is minimal misalignment of the chromatid.

A chromosome gap is a non-staining region (achromatic lesion) at the same locus in both chromatids of a single chromosome in which there is a minimal misalignment of the chromatids.

12.2 Chromatid breaks (b) and chromosome break (ib)

A chromatid break is a discontinuity of a single chromatid in which there is a clear misalignment of one of the chromatids.

Alternatively, the chromatid may be broken so that the broken fragment is displaced. In some cases, the fragment is not seen at all. A chromatid fragment (f) is scored if the chromosome of origin cannot be identified. In addition, deletions can occur as a result of a break. The missing terminal end of a chromatid in the assessed metaphase is classified as deletion (d).

A chromosome break is a discontinuity at the same locus in both chromatids of a single chromosome, giving rise to an acentric fragment with two chromatids and an abnormal monocentric chromosome.

A chromosome break is scored if the fragment is associated with a chromosome from which it was probably derived. However, fragments are often seen in isolation and are then scored as chromatid fragments (if). In addition, isodeletions can occur as a result of a isobreak. The missing terminal end of a chromosome in the assessed metaphase is classified as isodeletion (id).

The chromatid may remain aligned but show a gap which is too large to classify as a gap.

12.3 Chromatid exchanges (ex) and chromosome exchange (cx)

A chromatid exchange is the result of two or more chromatid lesions and the subsequent rearrangement of chromatid material. Exchanges may be between chromatids of different chromosomes or between or within chromatids of one chromosome. Chromatid exchanges have numerous different forms but are generally not further classified. Where multiple exchanges have occurred each exchange point is counted as one chromatid exchange.

A chromosome exchange is the result of two or more chromosome lesions and the subsequent relocation of both chromatids of a single chromosome to a new position on the same or on another chromosome. Chromosome exchanges generally appear as either a dicentric or a ring form, either of which can be associated with a fragment, which if possible should be scored as part of the exchange.

12.4 Multiple Aberrations

If many aberrations are present in one metaphase, the exact details may not be scorable. This is particularly the case when chromosome pulverisation (cd) occurs. If the number of aberrations is greater than 4, the cell is classified as multiple aberrant (ma).

12.5 Polyploid Cells / Endoreduplication

If the chromosome (centromer) number is 46 ±1 then it is classified as a diploid cell and scored for aberrations. If less than 46±1 chromosomes are counted then the cell is ignored under the assumption that some chromosomes may have been lost for technical reasons. If multiple copies of the haploid chromosome number (other than diploid) are scored then the count is recorded and the cell classified as polyploid. If the chromosomes are arranged in closely apposed pairs, i.e. 4 chromatids instead of 2, the cell is scored as endoreduplicated (e).

13 ANNEX 2: EXPERIMENT I – DETAILED DATA

13.1 Aberrations

For the analysis of chromosomal aberrations, 100 well spread metaphases per culture were scored. In the following tables, structural chromosome aberrations which were determined in treatments Experiment I without S9 mix and with S9 mix are given.

Table 13.1-a Experiment I without S9, exposure period 4 hours, preparation interval 22 hours, exposure date [redacted]

Culture	% Aberrant cells			Aberrations											
	incl. gaps	excl. gaps	with exchange	Gaps		Chromatid type				Chromosome type				Other	
				g	ig	b	f	d	ex	ib	if	id	cx	ma	cd
Solvent control: 0.5% culture medium without FCS without S9															
1	7	2	0	4	1	2	0	0	0	0	0	0	0	0	0
2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1+2	4	1	0	5	1	2	0	0	0	0	0	0	0	0	0
Positive control: 600 µg/mL EMS without S9															
1	37	33	15	4	0	25	0	0	23	2	2	0	0	0	0
2	45	44	22	1	0	32	1	1	28	3	1	0	0	1	0
1+2	41	38.5	18,5	5	0	57	1	1	51	5	3	0	0	1	0
Test item: 5000 µg/mL [redacted] without S9															
1	5	3	0	1	1	2	1	0	0	0	0	0	0	0	0
2	2	1	0	1	0	0	0	0	0	1	0	0	0	0	0
1+2	3.5	2	0	2	1	2	1	0	0	1	0	0	0	0	0
Test item: 2500 µg/mL [redacted] without S9															
1	5	0	0	5	0	0	0	0	0	0	0	0	0	0	0
2	4	2	0	2	0	2	0	0	0	0	0	0	0	0	0
1+2	4.5	1	0	7	0	2	0	0	0	0	0	0	0	0	0
Test item: 1250 µg/mL [redacted] without S9															
1	5	1	0	4	0	1	0	0	0	0	0	0	0	0	0
2	3	0	0	3	0	0	0	0	0	0	0	0	0	0	0
1+2	4	0.5	0	7	0	1	0	0	0	0	0	0	0	0	0

Abbrevations: g = gap, ig = iso-gap, b = break, ib = iso-break, f = fragment, if = isofragment, d = deletion, id = iso-deletion, ma = multiple aberration, ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (pulverisation)

Table 13.1-b Experiment I with S9, exposure period 4 h, preparation interval 22 hours, exposure date [redacted]

Culture	% Aberrant cells			Aberrations											
	incl. gaps	excl. gaps	with exchange	Gaps		Chromatid type				Chromosome type				Other	
				g	ig	b	f	d	ex	ib	if	id	cx	ma	cd
Solvent control: 0.5% culture medium without FCS with S9															
1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
2	3	0	0	3	0	0	0	0	0	0	0	0	0	0	0
1+2	2	0.5	0	3	0	1	0	0	0	0	0	0	0	0	0
Solvent control of the positive control: 0.5% NaCl (0.9%) with S9															
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	3	2	0	1	0	2	0	1	0	0	0	0	0	0	0
1+2	1.5	1	0	1	0	2	0	1	0	0	0	0	0	0	0
Positive control: 35 µg/mL CPA with S9															
1	38	26	5	12	7	26	3	0	5	0	1	0	1	0	0
2	54	46	7	17	3	53	0	0	5	0	1	0	4	2	0
1+2	46	36	6	29	10	79	3	0	10	0	2	0	5	2	0
Test item: 5000 µg/mL [redacted] with S9															
1	19	13	1	12	0	16	0	0	1	0	1	0	0	0	0
2	17	13	3	10	0	16	0	0	2	0	0	0	1	0	0
1+2	18	13	2	22	0	32	0	0	3	0	1	0	1	0	0
Test item: 2500 µg/mL [redacted] with S9															
1	20	12	3	11	0	11	0	0	3	0	1	0	1	0	0
2	5	2	0	3	0	1	0	0	0	0	1	0	0	1	0
1+2	12.5	7	1.5	14	0	12	0	0	3	0	2	0	1	1	0
Test item: 1250 µg/mL [redacted] with S9															
1	3	0	0	3	0	0	0	0	0	0	0	0	0	0	0
2	3	1	0	3	0	1	0	0	0	0	0	0	0	0	0
1+2	3	0.5	0	6	0	1	0	0	0	0	0	0	0	0	0

Abbrevations: g = gap, ig = iso-gap, b = break, ib = iso-break, f = fragment, if = isofragment, d = deletion, id = iso-deletion, ma = multiple aberration, ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (pulverisation)

14 ANNEX 3: EXPERIMENT II – DETAILED DATA

14.1 Aberrations

For the analysis of chromosomal aberrations, 100 well spread metaphases per culture were scored. In the following tables, structural chromosome aberrations which were determined in treatments Experiment II without S9 mix and with S9 mix are given.

Table 14.1-a Experiment II without S9, exposure period 22 hours, preparation interval 22 hours, exposure date [redacted]

Culture	% Aberrant cells			Aberrations											
	incl. gaps	excl. gaps	with exchange	Gaps		Chromatid type				Chromosome type				Other	
				g	ig	b	f	d	ex	ib	if	id	cx	ma	cd
Solvent control: 0.5% culture medium without FCS without S9															
1	6	4	0	1	1	3	0	0	0	0	1	0	0	0	0
2	3	1	0	2	0	0	0	0	0	1	0	0	0	0	0
1+2	4.5	2.5	0	3	1	3	0	0	0	1	1	0	0	0	0
Positive control: 300 µg/mL EMS without S9															
1	34	29	3	10	0	26	0	1	3	3	1	0	0	0	0
2	30	22	1	12	1	22	0	0	1	0	0	0	0	0	0
1+2	32	25.5	2	22	1	48	0	1	4	3	1	0	0	0	0
Test item: 4946 µg/mL [redacted] without S9															
1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0
2	2	1	0	1	0	1	0	0	0	0	0	0	0	0	0
1+2	1.5	1	0	1	0	1	0	0	0	0	1	0	0	0	0
Test item: 2473 µg/mL [redacted] without S9															
1	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0
2	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
1+2	1.5	0.5	0	2	0	1	0	0	0	0	0	0	0	0	0
Test item: 1236 µg/mL [redacted] without S9															
1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1+2	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0

Abbrevations
g = gap, ig = iso-gap, b = break, ib = iso-break, f = fragment, if = isofragment, d = deletion, id = iso-deletion, ma = multiple aberration, ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (pulverisation)

Table 14.1-b Experiment II with S9, exposure period 4 hours, preparation interval 22 hours, exposure date [redacted]

Culture	% Aberrant cells			Aberrations											
	incl. gaps	excl. gaps	with exchange	Gaps		Chromatid type				Chromosome type				Other	
				g	ig	b	f	d	ex	ib	if	id	cx	ma	cd
Solvent control: 0.5% culture medium without FCS with S9															
1	6	1	0	5	0	1	0	0	0	0	0	0	0	0	0
2	5	1	0	4	0	1	0	0	0	0	0	0	0	0	0
1+2	5.5	1	0	9	0	2	0	0	0	0	0	0	0	0	0
Solvent control of the positive control: 0.5% NaCl (0.9%) with S9															
1	3	1	0	2	0	0	0	0	0	1	0	0	0	0	0
2	3	0	0	3	0	0	0	0	0	0	0	0	0	0	0
1+2	3	0.5	0	5	0	0	0	0	0	1	0	0	0	0	0
Positive control: 35 µg/mL CPA with S9															
1	39	35	4	4	4	36	1	0	4	2	1	0	0	0	0
2	49	41	7	12	5	50	0	0	5	2	2	0	2	0	0
1+2	44	38	5.5	16	9	86	1	0	9	4	3	0	2	0	0
Test item: 4946 µg/mL [redacted] with S9															
1	42	39	9	5	0	43	0	1	5	0	0	0	5	1	0
2	29	25	8	7	2	26	0	0	7	0	0	0	3	1	0
1+2	35.5	32	8.5	12	2	69	0	1	12	0	0	0	8	2	0
Test item: 2473 µg/mL [redacted] with S9															
1	12	10	0	5	0	12	0	1	0	0	0	0	0	0	0
2	22	16	2	9	0	17	0	0	1	0	0	0	1	1	0
1+2	17	13	1	14	0	29	0	1	1	0	0	0	1	1	0
Test item: 1236 µg/mL [redacted] with S9															
1	2	1	0	1	0	1	0	0	0	0	0	0	0	0	0
2	3	1	0	3	0	2	0	0	0	0	0	0	0	0	0
1+2	2.5	1	0	4	0	3	0	0	0	0	0	0	0	0	0
Test item: 618 µg/mL [redacted] with S9															
1	5	1	0	4	0	1	0	0	0	0	0	0	0	0	0
2	3	1	0	2	0	1	0	0	0	0	0	0	0	0	0
1+2	4	1	0	6	0	2	0	0	0	0	0	0	0	0	0

Abbrevations see table on previous page

15 ANNEX 4: BIOMETRY

15.1 Equation

Statistical evaluation was made following Richardson, C., Williams, D.A., Allen, J., Amphlett, G.E., Chanter, D.O., and Phillips,B. (1988), Analysis of data from in vitro cytogenetic assays, In Statistical Evaluation of Mutagenicity Test Data (ed D,J, Kirkland), 141-154, Cambridge University Press, Cambridge. The following equation was used for the calculation of the p of each distribution:

$$\varphi(a) = \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{n!a!b!c!d!}$$

The resulting probability of the respective distribution was halved and the probabilities of the more extreme distributions (down to a value of 0 in the controls) were added to give the cumulated p-value of the tail of the distribution.

15.2 Biometry of Experiment I

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of Fisher's exact test. Evaluation was performed only for cells carrying aberration excluding gaps.

Table 15.2-a Biometry of Experiment I

Concentration / test group	Aberrant cells in % exclusive gaps	p-value
Experiment I: exposure period 4 hrs without S9, exposure dat[redacted]		
Solvent control: 0.5% (v/v) Culture medium without FCS	1.0	--
Positive control: 600 µg/mL EMS	38.5[S]	< 0.0001
Test item: 4940 µg/mL[redacted]	2.0	0.225
Test item: 2470 µg/mL[redacted]	1.0	n.c.
Test item 1240 µg/ml[redacted]	0.5	n.c.
Experiment I: exposure period 4 hrs with S9, exposure date[redacted]		
Solvent control 0.5% (v/v) Culture medium without FCS	0.5	--
Solvent control of the positive control: 0.5% NaCl (0.9%)	1.0	--
Positive control: 35 µg/mL CPA	36.0[S]	< 0.0001
Test item: 4940 µg/mL[redacted]	13.0[S]	< 0.0001
Test item: 2470 µg/mL[redacted]	7.0[S]	< 0.001
Test item 1240 µg/mL[redacted]	0.5	n.c.

n.c.: not calculated as the aberration rate is equal or lower than the control rate
[S]: Aberration rate is statistically significant higher than the control rate

15.3 Biometry of Experiment II

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of Fisher's exact test. Evaluation was performed only for cells carrying aberration excluding gaps.

Table 15.3-a Biometry of Experiment II

Concentration / test group	Aberrant cells in % exclusive gaps	p-value
Experiment II: exposure period 4 hrs without S9, exposure date [redacted]		
Solvent control: 0.5% (v/v) Culture medium without FCS	2.5	--
Positive control: 300 µg/mL EMS	25.5[S]	< 0.0001
Test item: 4940 µg/mL [redacted]	1.0	n.c.
Test item: 2470 µg/mL [redacted]	0.5	n.c.
Test item 1240 µg/mL [redacted]	0	n.c.
Experiment II: exposure period 4 hrs with S9, exposure date [redacted]		
Solvent control: 0.5% (v/v) Culture medium without FCS	1.0	--
Solvent control of the positive control: 0.5% NaCl (0.9%)	0.5	--
Positive control: 35 µg/mL CPA	38.0[S]	< 0.0001
Test item: 4940 µg/mL [redacted]	32.0[S]	< 0.0001
Test item: 2470 µg/mL [redacted]	13.0[S]	< 0.0001
Test item: 1240 µg/mL [redacted]	1.0	n.c.
Test item 620 µg/mL [redacted]	1.0	n.c.

n.c. Not calculated as the aberration rate is equal or lower than the control rate
[S] Aberration rate is statistically significant higher than the control rate

16 ANNEX 5: HISTORICAL LABORATORY CONTROL DATA

16.1 Percentage of Aberrant Cells in Human Lymphocytes

Table 16.1-a Historical Aberrant Cells

Test group Concent-ration	Cells scored	Aberrant cells in %								
		Inclusive gaps			Exclusive gaps*			with exchanges		
		Range	Mean	Calcu. range*	Range	Mean	Calcu. range*	Range	Mean	Calcu. ran-ge*
Without S9										
Solvent control A.dest 0.5% (v/v)	2800	0.0-5.0	2.3	0.6-4.0	0.0-2.5	0.9	-0.2-1.9	0.0-0.5	0.0	-0.1-0.2
Solvent control DMSO 0.5% (v/v)	400	0.0-5.0	2.5	-1.0-6.0	0.0-1.0	0.5	-0.2-1.2	0.0-0.0	0.0	0.0-0.0
Solvent control Medium	800	1.5-4.0	3.0	1.9-4.1	0.0-2.5	1.3	0.2-2.3	0.0-0.0	0.0	0.0-0.0
Positive control EMS 300-600 µg/ml	4000	18.0-43.0	31.9	24.1-39.6	15.5-39.5	26.9	20.2-33.5	1.5-20.0	5.1	1.2-9.0
With S9										
Solvent control A.dest 0.5% (v/v)	2800	0.0-5.0	2.3	0.8-3.8	0.0-3.5	1.3	0.2-2.3	0.0-0.0	0.0	0.0-0.0
Solvent control DMSO 0.5% (v/v)	400	0.1-2.0	1.1	-0.3-2.4	0.1-2.0	1.1	-0.3-2.4	0.0-0.0	0.0	0.0-0.0
Solvent control Medium	800	0.5-3.0	2.1	1.0-3.2	0.5-1.5	1.0	0.6-1.4	0.0-0.0	0.0	0.0-0.0
Positive control CPA 35 µg/ml	4000	21.5-47.5	37.5	29.6-45.4	16.5-42.5	31.0	23.5-38.5	0.0-9.0	3.9	1.5-6.3

* Calculated range = Mean +/- standard deviation

17 ANNEX 6: GLP-CERTIFICATE

RheinlandPfalz
LANDESAMT FÜR UMWELT, WASSERWIRTSCHAFT UND GEWERBEAUFSICHT

GUTE LABORPRAXIS – GOOD LABORATORY PRACTICE

GLP-BESCHEINIGUNG
STATEMENT OF GLP COMPLIANCE

gemäß/according to § 19b Abs. 1 Chemikaliengesetz

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EC at:

Prüfstandort / Test site

[redacted]

Prüfung nach Kategorien / Areas of Expertise
(gemäß / according ChemVwV-GLP Nr. 5.3/OECD guidance)
9 (Durchführung zytogenetischer Untersuchungen „in vitro" als Phase toxikologischer Prüfungen)

Datum der Inspektion / Date of Inspection
(Tag.Monat.Jahr / day.month.year)
22.06.2011

Der genannte Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in diesem Prüfstandort die oben genannten Phasen von Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that the test site is able to conduct the aforementioned phases of studies in compliance with the Principles of GLP.

Eine erneute behördliche Überprüfung der Einhaltung der GLP-Grundsätze durch den Prüfstandort ist spätestens drei Jahre nach der letzten Inspektion zu beantragen. Ohne diesen Antrag wird der Prüfstandort nach Ablauf der Frist aus dem deutschen GLP-Überwachungsprogramm genommen und diese GLP-Bescheinigung verliert ihre Gültigkeit.

Verification of the compliance of the test site with the Principles of the GLP has to be applied for not later than three years after the last inspection. Elapsing this term, the test site will be taken out of the German GLP-Monitoring Programme and this GLP Certificate becomes invalid.

Unterschrift, Datum / Signature, Date

9.12.2011

Dr.-Ing. Pia Hirsch - Stellvertretung Präsident
(Name und Funktion der verantwortlichen Person / name and function of responsible person)

Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht
Kaiser-Friedrich-Straße 7, 55116 Mainz
(Name und Adresse der GLP-Überwachungsbehörde / Name and adress of the GLP Monitoring Authority)

MESSEN BEWERTEN BERATEN